WHAT IS CLAIMED IS:

1. A method for producing multi-gene recombinant vector constructs, which comprises:

(1) a multi-gene assembly vector system comprising an acceptor vector and at least two donor vectors; and

(2) a DNA recombination system allowing two or more rounds of gene assembly by sequential DNA delivery into the acceptor vector via DNA swapping between the acceptor vector and different donor vectors; and multiple donor vectors will be rotatively used in different rounds of recombination to allow sequential insertion of genes or DNA fragments into the acceptor vector.

2. The method of claim 1, wherein said acceptor vector comprises:

(1) a site RS for DNA recombination;

(2) a site S1 located near said RS, which is a cutting site for a homing endonuclease or a restriction endonuclease, or a site for irreversible specific recombination;

(3) a selection marker gene that is different from that contained in said donor vector; and

(4) a replicon for replication, including those capable of maintaining large plasmids.

3. The method of claim 1, wherein a donor vector I comprises:

(1) a site RS for DNA recombination, which is the same site RS as on said acceptor vector or can form a specific recombination with the RS on said acceptor vector;

(2) a site S1 and another site S2, which are cutting sites for homing

endonucleases, or for rare-cutting restriction endonucleases, or sites for irreversible recombination;

(3) a multi-cloning site MCS;

(4) locations of the sites of RS, S1, S2 and MCS on said donor vector in relative order of RS-S2-MCS-S1; and

(5) a selection marker gene different from that contained in said acceptor vector.

4. The method of claim 1, wherein another donor vector II comprises:

(1) a site RS for DNA recombination, which is the same site RS as on said acceptor vector or can form a specific recombination with the RS on said acceptor vector;

(2) a site S1 and another site S2, which are cutting sites for homing endonucleases, or for rare-cutting restriction endonucleases, or sites for irreversible recombination;

(3) a multi-cloning site MCS;

(4) locations of the sites of RS, S1, S2 and MCS on said donor vector in relative order of RS-S1-MCS-S2; and

(5) a selection marker gene different from that contained in said acceptor vector.

5. The method according to claim 1, wherein said multi-gene assembly vector system comprising an acceptor vector and donor vector I and donor vector II is used to carry out two or more cycles of DNA recombination by alternate use of said donor vector I and donor vector II together with said acceptor vector to construct multi-gene vector constructs, which recombination process comprises

the steps of:

(1) cloning of target single genes or gene groups of interest including any DNA fragments by conventional molecular cloning techniques into the MCS of said donor vector I or donor vector II, to make the target gene or genes inserted between the sites S1 and S2;

(2) carrying out the first cycle of DNA recombination to recombine the first target gene or gene groups into said acceptor vector through: (i) *in vivo* or *in vitro* plasmid recombination with said donor vector I containing target gene and said acceptor vector, upon double-selection of transformants with the selection marker genes of the plasmids; (ii) removing the backbone sequence of said donor vector I between the two S1 sites from the integrative plasmid by digestion with an endonuclease that cut S1 sites, followed by plasmid circularization by ligation with T4 DNA ligase, and if necessary, with the aid of a double-stranded oligonucleotide linker compatible to the S1 cutting ends; and (iii) if S1 is an irreversible recombination site, performing an *in vivo* or *in vitro* recombination reaction with corresponding recombinase, upon which the backbone sequence of said donor vector I between the two S1 sites is removed and the ends of the acceptor vector bearing the inserted gene or gene group are joined to form a circular plasmid;

(3) carrying out the second cycle of DNA recombination to recombine the second target gene or gene groups into said acceptor vector through: (i) *in vivo* or *in vitro* plasmid recombination with said donor vector II containing the second gene or gene group and the acceptor vector plasmid obtained from the step (2), upon double-selection of transformants with the selection marker genes

of the plasmids; (ii) removing the backbone sequence of the donor vector II between the two S2 sites from the integrative plasmid by digestion with an endonuclease that cut S2 sites followed by plasmid circularization by ligation with T4 DNA ligase, and if necessary, with the aid of a double-stranded oligonucleotide linker compatible to the S2 cutting ends; and (iii) if S2 is an irreversible recombination site, performing an *in vivo* or *in vitro* recombination reaction with corresponding recombinase, upon which the backbone sequence of the donor vector II between the two S2 sites is removed and the ends of the acceptor vector bearing the inserted genes or gene groups are joined to form a circular plasmid;

(4) repeating said step (2) and step (3) with alternate donor vector plasmids containing target gene or gene group and the acceptor vector plasmid obtained in the former step, until all target genes or DNA fragments being linked into the acceptor vector to finish a designed vector construct.

6. An acceptor vector plasmid according to claim 1, which comprises all or part of the components shown in Fig. 1A or all or part of the DNA sequence SEQ ID NO: 1.

7. A donor vector I plasmid according to claim 1, which comprises all or part of the components shown in Fig. 1B or all or part of the DNA sequence SEQ ID NO: 2.

8. A donor vector II plasmid according to claim 1, which comprises all or part of the components shown in Fig. 1C or all or part of the DNA sequence SEQ ID NO: 3.

9. The application of the method of claim 1, wherein multiple genes or

DNA fragments of interest are combined to a vector to create a desired vector construct, or the genes combined in the vector construct are transferred together into selected recipients to obtain multiple gene-products or express multi-gene-depended characters.